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13. ABSTRACT *(Maximum 200 words)*

Our objective was to design an *in situ* instrument to analyze the optical properties of individual suspended particles, which are important to ocean geochemistry, optics, and biology. The approach arrived at is that of a flow cytometer modified to facilitate in situ unattended operation (use of a battery-powered solid state laser for fluorescence excitation, and of a neural network to carry out real-time data analysis). To increase reliability we use a simple ducted flow of seawater through the instrument rather than injection of seawater into the center of a particle-free fluid stream as in conventional flow cytometers. Simple ducted flow, which does not mechanically confine sample particles to a path through the central region of the focussed laser beam, requires a means of discriminating particles with acceptable trajectories from those which pass through the less-intense edges of the beam and thus give inaccurate signals. We have demonstrated that two orthogonal infrared diode laser beams can be used for this purpose: the beams can be arranged so that particles passing through both infrared beams must also pass through the center of the fluorescence excitation beam, and signals from only these particles will be collected. The instrument is now in development.

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Final Report, ONR grant N00014-93-1-1171, Feasibility of an in situ flow cytometer

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Summary of Project

This project's objective was to evaluate designs for an *in situ* instrument to analyze the optical properties of individual suspended particles, which are important to ocean geochemistry, optics, and biology. The approach arrived at is that of a flow cytometer modified to facilitate in situ unattended operation (use of a battery-powered solid state laser for chlorophyll and phycoerythrin fluorescence excitation, and of a neural network to carry out real-time data analysis). To increase reliability we use a simple ducted flow of seawater through the instrument rather than injection of seawater into the center of a particle-free fluid stream as in conventional flow cytometers. Simple ducted flow, which does not mechanically confine sample particles to a path through the center of the focussed laser beam, requires a means of discriminating particles with acceptable trajectories from those which pass through the less-intense edges of the beam and thus give inaccurate signals. We have demonstrated that two orthogonal infrared diode laser beams can be used for this purpose: the beams can be arranged so that particles passing through both infrared beams must also pass through the center of the fluorescence excitation beam, and signals from only these particles will be collected. The instrument is now in development.

This project's objective was to evaluate designs and arrive at a plan for constructing an *in situ* instrument to analyze the optical properties of individual suspended particles in the ocean. The resulting design was presented as a poster in the Oceanographic Instrumentation session at the AGU Ocean Sciences meeting in February 1996, and we are now in the process of constructing an instrument based on the results of this project (with funding from DOE and NSF). We analyzed the proposed approach using a laboratory mockup system, some of whose results are shown below. We plan to use an intermediate version of the instrument in ONR's Coastal Mixing and Optics project starting this summer.

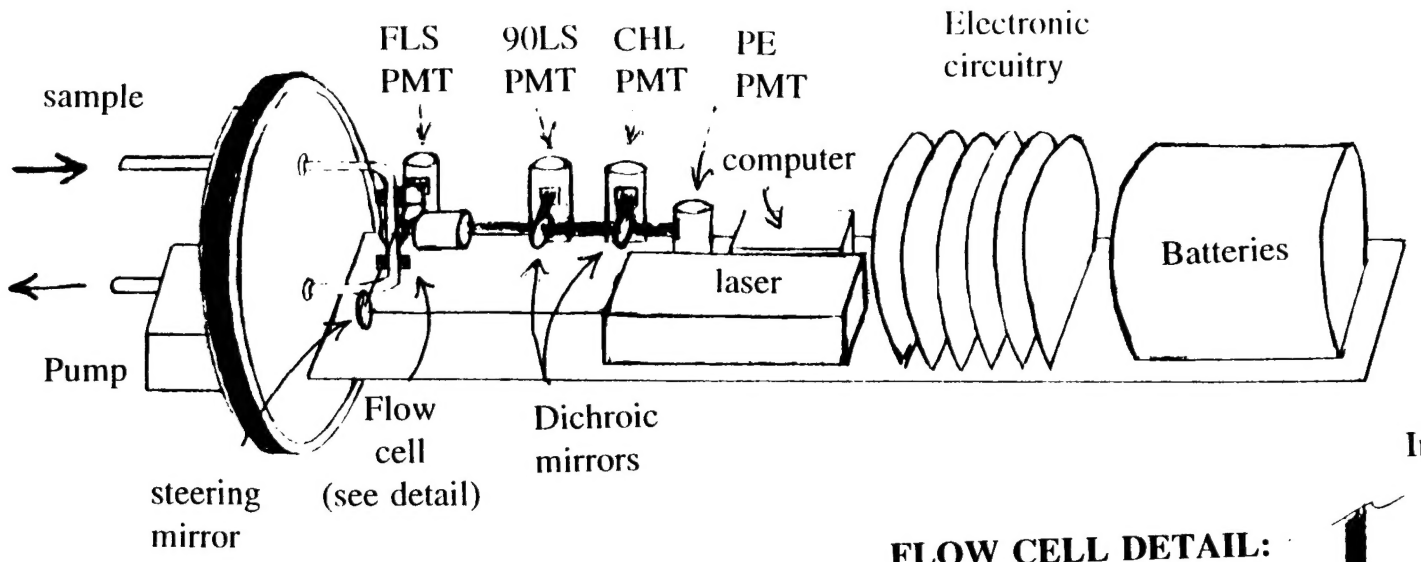
Our starting point was a flow cytometer, with which we have experience in making quantitative and rapid measurements of light scattering and fluorescence on individual phytoplankton cells. However, there are problems to be addressed before this laboratory instrument could succeed in unattended in situ operation. The first of these is the electrical power requirement of the laser used to excite fluorescence, which in conventional flow cytometers is typically hundreds to thousands of watts, far more than is possible to supply by batteries (during moored operation, for example). With the availability of solid state laseres (such as diode-pumped frequency-doubled Nd-YAG lasers), it is now possible to obtain adequate power for fluorescence excitation (e.g., 100 mW at 532 nm) at relatively low power cost. An instrument based on such a laser could be self-contained and battery-powered (Fig. 1).

A second problem is that for in situ unattended operation, an increased degree of mechanical reliability will be required. To avoid the need for injection of seawater into the center of a particle-free fluid stream as in conventional flow cytometers, we will use a simple ducted flow of seawater through the instrument. Simple ducted flow, which does not physically force sample particles through the uniform central region center of the focussed laser beam, requires a means of discriminating particles with acceptable trajectories from those which pass through the non-uniform edges of the beam and thus give inaccurate signals. We have demonstrated that two orthogonal infrared (IR) diode laser beams can be used for this purpose; with appropriate focussing, particles passing through both infrared beams will also pass through the center of the fluorescence excitation beam, and signals from only these particles will be collected (Figs. 2, 3, 4).

Laboratory tests indicated that IR diode laser beams can be focussed and aimed with sufficient precision to define an analysis region within the uniform central region of a Gaussian measuring beam. This test was first performed using a conventional flow cytometer sample injection system to provide a core stream containing fluorescent beads: it was shown that the core could be displaced far enough to eliminate IR-scattering signals without significantly affecting the signals from the main beam (i.e., the IR beam was detecting only those beads passing through the center of the main beam). In addition, when beads were added to the bulk fluid and simple ducted flow was used with a prototype detection and logic circuit, small signals (i.e., those from beads passing through the edges of the main laser beam) were selectively eliminated (Fig. 5).

A third problem, that of the large amount of data obtained from continuous multiparameter measurements of individual particles, will be addressed through use of a neural network real-time data analysis approach. By producing statistical summaries and single-parameter distributions for a limited number of particle categories, data accumulation can be kept to a reasonable level even during a long-term deployment.

The instrument is now in development.



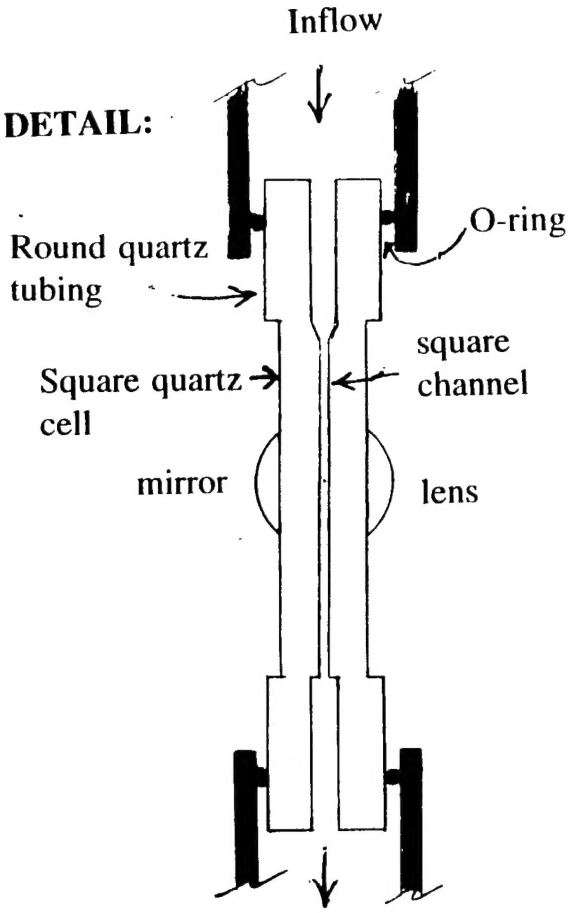


Figure 1. Schema of *in situ* flow cytometer. The instrument's optical table is mounted to the end cap of a cylindrical housing. Seawater is pulled through the flow cell (expanded view) inside the pressure housing by a pump on the outflow end of a narrow tube. The flow cell is square, but is fused to round quartz tube on the ends to facilitate sealing by O-rings. To save space the laser beam is steered with mirrors to the center of the flow cell channel, at the focal point of the collection optics. Beam shaping optics are not shown here.

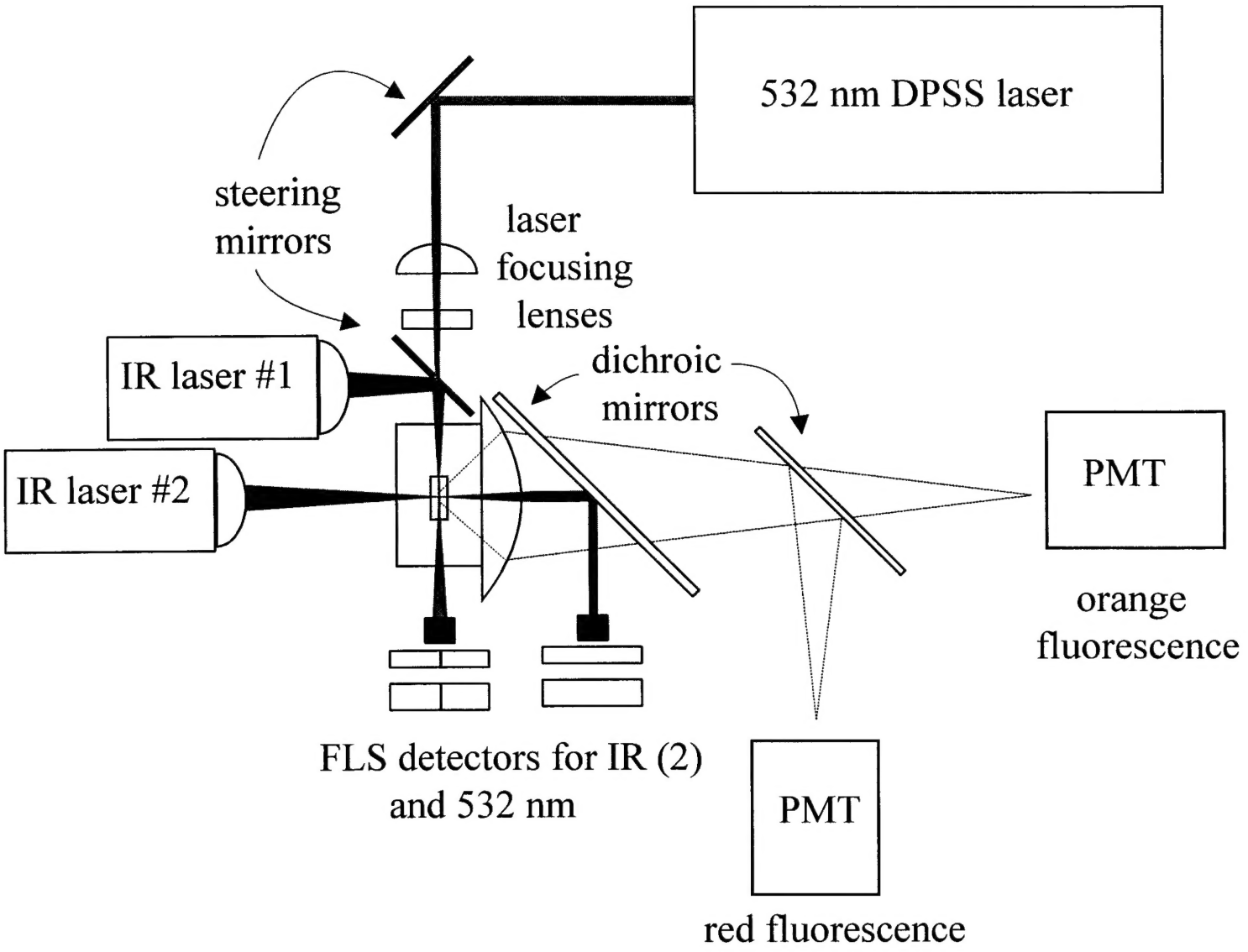


Fig. 2. Schema of the optical arrangement for the *in situ* flow cytometer, as viewed from above. A green (532-nm) beam from a frequency-doubled diode-pumped solid-state (DPSS) Nd:Yag laser is focussed on the center of the flow cell channel (180x400 μm), just as in a conventional flow cytometer. Seawater containing phytoplankton and other particles is pulled through the flow cell by a pump on the outflow side, producing laminar flow in the center of the cell.

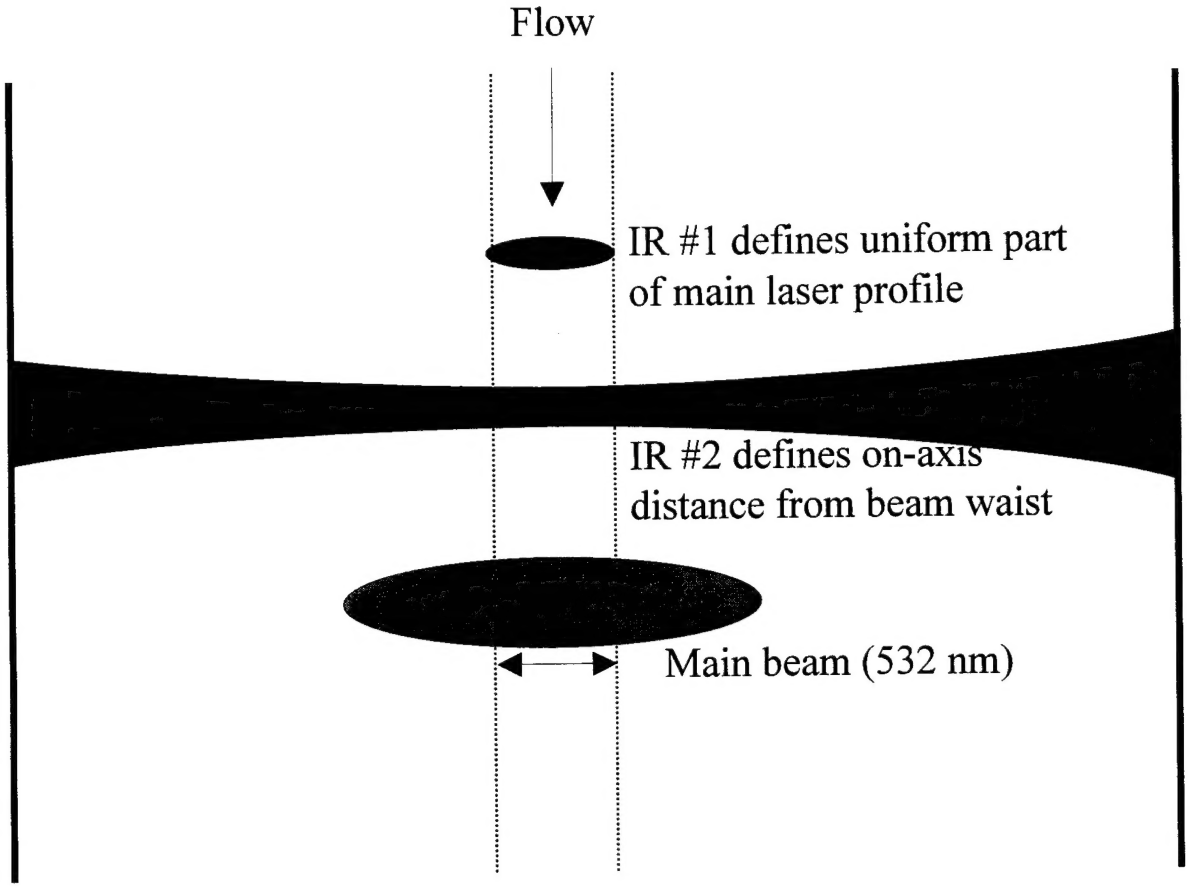
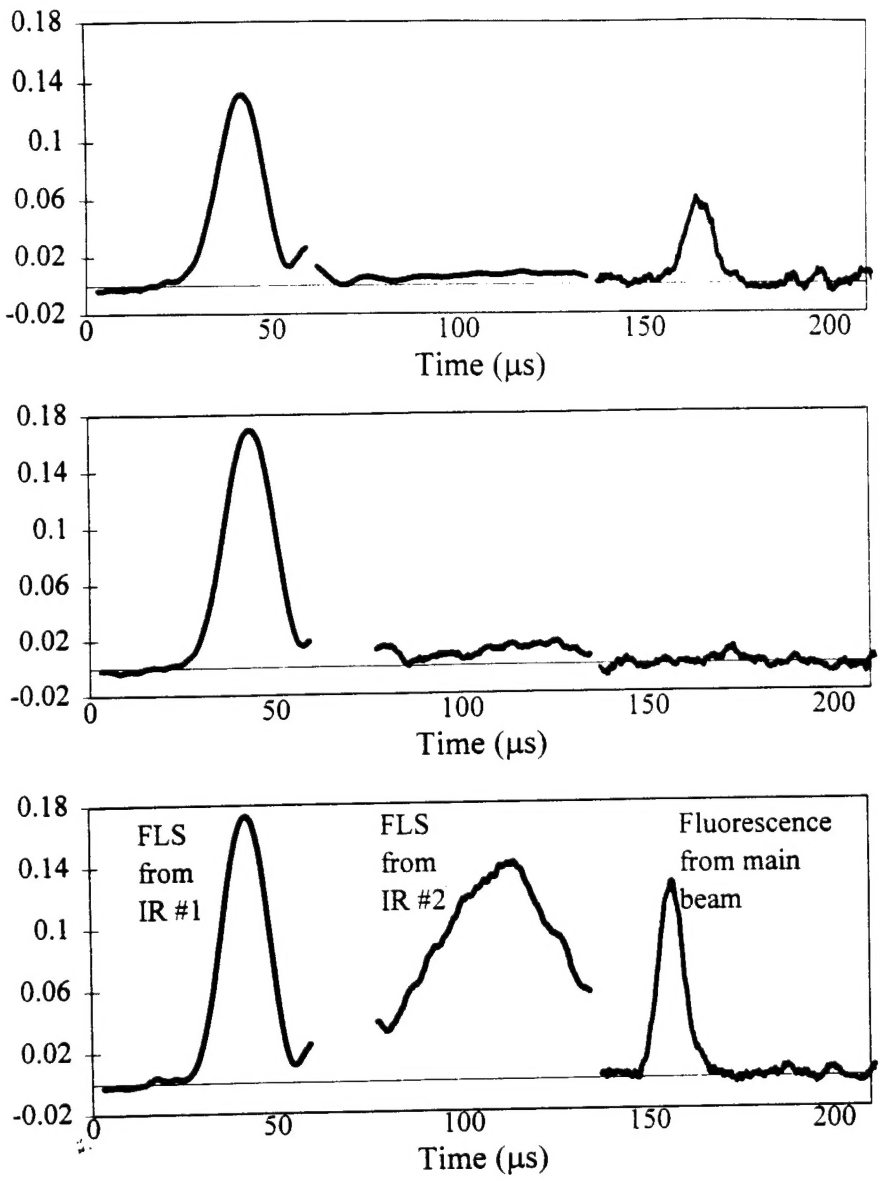


Fig. 3. Schema of flow cell channel and laser beams, viewed from axis of main beam (green)

Two infrared (IR) diode lasers focused slightly upstream are used to detect the approach of particles that will pass through the uniform region of the green beam. One of the IR beams is nearly parallel to the main green beam, and its beam waist is focussed to a spot smaller than that of the main beam to define the central, uniform part of the main beam. The laser intensity also decreases with distance away from the beam waist on-axis; IR beam #2, oriented perpendicular to the main beam, defines an along-axis region of acceptable uniformity.



(3) A particle traversing only one IR beam may hit the main beam far from the beam waist, giving a small signal which should be rejected.

(2) A particle passing through only one IR beam may miss the main beam and will not be "acquired".

(1) A particle traversing both IR beams will also pass through the center of main beam, allowing accurate measurements.

Fig. 4. Testing the optical definition of the sensing region.
The two IR lasers were aligned to the main beam while analyzing fluorescent plastic microspheres (5.2 mm diameter) using a conventional sample injection system with distilled water as sheath fluid. Signals from the three detectors (two IR forward scattering detectors and red fluorescence excited by the main beam) were displayed using a digital oscilloscope, and the positions of the beams were adjusted so that beads traversed all three beams. Then the sample pump was stopped and beads were added to the sheath reservoir, so that beads were introduced in the bulk flow. The oscilloscope was set to trigger on signals from the first IR beam, and the results of 300 events were stored. Three kinds are shown:
(1) When both IR signals were present, fluorescence signals were also.
(2) When the second IR signal was not present, fluorescence was usually absent as well; when a fluorescence signal was present **(3),** it was usually small.

For quantitative measurements of particle size and fluorescence, we should only measure signals from case (1) events.

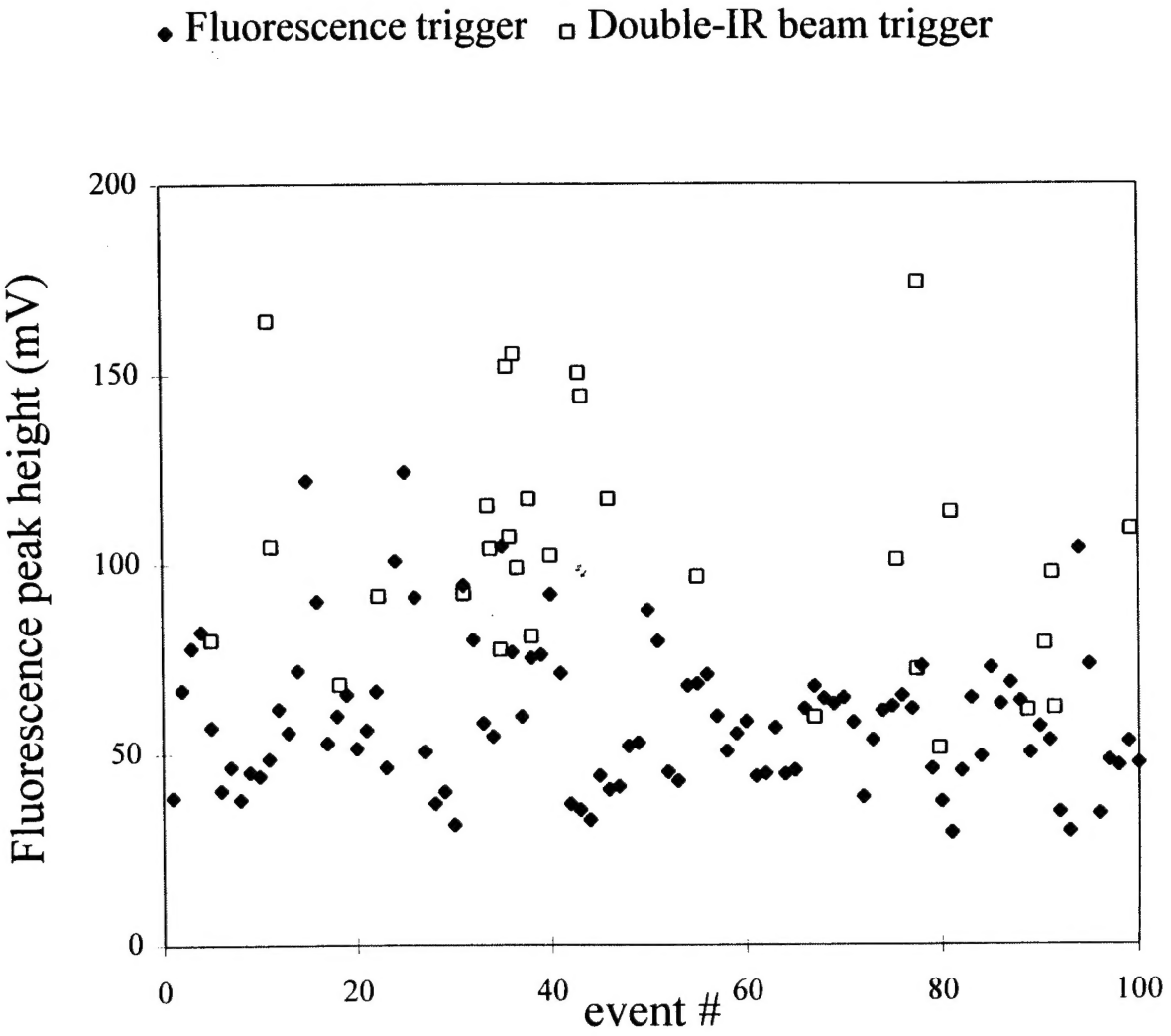


Fig. 5. Of the beads triggering the first IR beam in Fig. 4, about 10% also triggered the second IR beam, and thus produced "acceptable" fluorescence signals. When these were compared to 100 signals triggered directly from fluorescence signals (i.e., no selection), it is obvious that the "IR-selected" signals are much larger than the unselected ones. However, the distribution of signal sizes is much broader than expected for these beads (C.V.>15% vs 5% expected), and some small signals were acquired. These results suggest that the focus and/or position of the IR beams was not sufficiently precise to correctly define the sensing region in this first trial.